--Abstract of the Disclosure

The disclosure describes recombinant alphavirus RNA molecules and expression of heterologous proteins therefrom in animal cells. Recombinant alphaviruses of the present invention, when made to express an antigenic protein, can be administered as vaccines.--

In the Claims:

Please delete claims 1-41 without prejudice to or disclaimer of the subject matter contained therein.

Please add the following new claims.

--42. A helper cell for producing an infectious, defective alphavirus particle, comprising, in an alphavirus-permissive cell:

(a) an alphavirus replicon RNA, wherein the alphavirus is selected from the group consisting of Sindbis virus and Semliki Forest virus; wherein the replicon RNA comprises the alphavirus packaging signal, a heterologous RNA sequence, and a sequence encoding at least one of the alphavirus structural proteins, wherein the replicon RNA furthermore lacks a sequence encoding at least one of the alphavirus structural proteins; and

(b) at least one separate helper RNA encoding the structural protein(s) absent from the replicon RNA, said helper RNA(s) lacking the alphavirus packaging signal;

wherein the combined expression of the replicon RNA and the helper RNA(s) produces an assembled alphavirus particle which comprises a heterologous RNA sequence, is able to infect a cell, and is unable to complete viral replication in the absence of helper RNA due to the absence of the structural protein coding sequence in the packaged replicon.

43. The helper cell according to claim 42, wherein said replicon RNA encodes the alphavirus capsid protein, and wherein said at least one separate helper RNA(s) encodes the alphavirus E1 glycoprotein and the alphavirus E2 glycoprotein.

44. The helper cell according to claim 42, wherein said alphavirus is Venezuelan Equine Encephalitis virus.

45. The helper cell according to claim 42, wherein at least one of said helper RNA and said replicon RNA includes at least one mutation in E1, E2 or E3.

46. The helper cell according to claim 42, wherein said alphavirus is Semliki Forest virus.

47. The helper cell according to claim 42, wherein said helper RNA and said replicon RNA both include a promoter.

48. The helper cell according to claim 42, wherein said replicon RNA includes a promoter.

49. The helper cell according to claim 42, wherein said inserted heterologous RNA is selected from the group consisting of RNA encoding proteins and RNA encoding peptides.

50. A method of making infectious, defective, alphavirus particles, comprising:

providing a helper cell according to claim 42;

producing said alphavirus particles in said helper cell; and

collecting said alphavirus particles from said cell.

51. The method according to claim 50, wherein said alphavirus replicon RNA and said at least one separate helper RNA are introduced into said helper cell by electroporation.

52. Infectious alphavirus particles produced by the method of claim 50.

53. A pharmaceutical formulation comprising infectious alphavirus particles according to claim 52 in an effective immunogenic amount in a pharmaceutically acceptable carrier.

54. A helper cell for producing an infectious, defective alphavirus particle, comprising an alphavirus-permissive cell transfected with RNAs comprising:

(a) an alphavirus replicon RNA, wherein the replicon RNA comprises the alphavirus packaging signal, a heterologous RNA sequence, wherein the replicon RNA furthermore lacks sequences encoding alphavirus structural proteins; and

(b) at least a first and second helper RNAs separate from said replicon RNA and separate from each other, said first and second helper RNAs encoding the structural proteins absent from the replicon RNA;

with said first helper RNA encoding at least one alphavirus structural protein and not encoding at least one other alphavirus structural protein;

with said second helper RNA not encoding said at least one alphavirus structural protein encoded by said

first helper RNA and encoding said at least one other alphavirus structural protein not encoded by said first helper RNA;

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and with said first and second helper RNAs lacking the alphavirus packaging signal;

wherein the combined expression of the replicon RNA and the helper RNAs produces an assembled alphavirus particle which comprises a heterologous RNA sequence, is able to infect a cell, and is unable to complete viral replication in the absence of the helper RNAs due to the absence of the structural protein coding sequence in the packaged replicon.

55. The helper cell according to claim 54, wherein said first helper RNA encodes both the alphavirus E**1** glycoprotein and the alphavirus E**2** glycoprotein, and wherein said second helper RNA encodes the alphavirus capsid protein.

56. The helper cell according to claim 54, wherein said first helper RNA and said second helper RNA both include a promoter.

57. The helper cell according to claim 54, wherein said replicon RNA includes a promoter.

58. The helper cell according to claim 54, wherein said inserted heterologous RNA is selected from the group consisting of RNA encoding proteins and RNA encoding peptides.

59. A method of making infectious, defective, alphavirus particles, comprising:

providing a helper cell according to claim 54;

producing said alphavirus particles in said helper cell; and then

collecting said alphavirus particles from said cell.

60. The method according to claim 59, wherein said alphavirus replicon RNA and said at least first and second helper RNAs are introduced into said helper cell by electroporation.

61. A cell expressing:

i) a first recombinant RNA molecule comprising an alphavirus RNA genome and an exogenous RNA sequence, wherein said alphavirus RNA genome contains a signal for packaging said recombinant RNA molecule in an alphavirus particle, at least one deletion or stop codon mutation such that at least one structural protein of the alphavirus cannot be translated from said recombinant RNA in said cell, and further wherein said exogenous RNA sequence is operatively inserted into a region of

the alphavirus RNA genome which is non-essential to replication of the recombinant RNA molecule; and

ii) a second recombinant RNA molecule encoding a said at least one structural protein of the alphavirus, wherein said second recombinant RNA molecule lacks a signal for packaging of said second recombinant RNA molecule in an alphavirus particle.

62. A method for producing a recombinant or chimeric alphavirus particle comprising:

i) providing a cell that produces a recombinant or chimeric alphavirus particle, said cell comprising:

a) a first recombinant RNA molecule comprising an alphavirus RNA genome and an exogenous RNA sequence, wherein said alphavirus RNA genome contains a signal for packaging said recombinant RNA molecule in an alphavirus particle, at least one deletion or stop codon mutation such that at least one structural protein of the alphavirus cannot be translated from said recombinant RNA in said cell, and further wherein said exogenous RNA sequence is operatively inserted into a region of the alphavirus RNA genome which is non-essential to replication of the recombinant RNA molecule; and

b) a second recombinant RNA molecule encoding a said at least one structural protein of the alphavirus, wherein said second

recombinant RNA molecule lacks a signal for packaging of said second recombinant RNA molecule in an alphavirus particle;

ii) culturing said cell to produce said recombinant or chimeric alphavirus particles; and

iii) recovering said recombinant or chimeric alphavirus particles from said culture.

63. A helper cell for producing an infectious, defective alphavirus particle, comprising, in an alphavirus-permissive cell:

(a) an alphavirus replicon RNA, wherein the alphavirus is selected from the group consisting of Sindbis virus and Semliki Forest virus; wherein the replicon RNA comprises the alphavirus packaging signal, a heterologous RNA sequence, and a sequence encoding at least one of the alphavirus structural proteins, wherein the replicon RNA furthermore lacks a sequence encoding at least one of the alphavirus structural proteins; and

(b) a helper RNA system comprising helper RNAs encoding the structural protein(s) whose transcripts are absent from or otherwise not functional in the replicon RNA, said helper RNA(s) lacking any alphavirus packaging signal;

wherein the combined expression of the replicon RNA and the helper RNA(s) produces an assembled alphavirus particle which comprises a heterologous RNA sequence, is able to infect a cell, and is unable to complete viral replication in the absence of helper RNA due to the absence of at least one structural protein coding sequence in the packaged replicon.--